IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

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Applicant	:	Anup Sood	
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Examiner	:	Steven C. Pohnert	
Docket No.	:	PB0313	
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April 14, 2008

APPEAL BRIEF

Sir:

Appellants submit this Appeal Brief, appealing from the September 14, 2007 final rejection, and the January 31, 2008 Advisory Action, of the Examiner, finally rejecting claims 1-56, in the captioned application. The Notice of Appeal was filed on January 14, 2008, which contained authorization to charge the "Appeal Fee" to Appellants' Deposit Account. Applicants are filing herewith a petition to extend the period for filing this brief one month from March 14, 2008 to April 14, 2008. Authorization to charge the required fee is included therein.

Real Party in Interest

GE Healthcare Bio-Sciences Corp., formerly known as Amersham Biosciences Corp., the assignee and owner of the captioned application, is the real party in interest to this appeal.

Related Appeals and Interferences

There are no other appeals or interferences related to the instant appeal.

Status of Claims

Claims 1-56 are pending in the captioned application. Claims 57-62 have been cancelled. Claims 1-56 are the only pending claims subject to examination before the U.S. Patent and Trademark Office. These claims are finally rejected and constitute the claims under appeal. A copy of these claims is appended hereto.

Status of Amendments

Claim 1 has been amended in a response to Final Rejection submitted to the Office on January 14, 2008. This amendment has been entered by Examiner in an Advisory Action dated January 31, 2008. There are no other outstanding amendments with regard to the captioned application.

Summary of Claimed Subject Matter

The instant invention relates to methods for sequencing a target region of a nucleic acid template. Claims 1 and 32 are the independent claims. The methods include a nucleic acid polymerization reaction, which reaction uses a terminal phosphate-labeled nucleoside polyphosphate and at least one component is

immobilized on a solid support. If the nucleoside base is complementary to the template base at the site of polymerization, a labeled polyphosphate is generated.

In claim 1, the reaction mixture is further treated with a phosphatase to produce a detectable species from the labeled polyphosphate, followed by detection of the detectable species. Different terminal-labeled nucleoside polyphosphates are used to identify additional sequences. (See page 4, line 20 to page 5, line 14). The terminal phosphate-labeled nucleoside polyphosphates are substantially non-reactive to the phosphatase (page 3, lines 26-28). The detectable species is subsequently detected without separation by charge from the other reaction components (page 4, lines 6-9; page 4, lines 12-14; and page 12, lines 1-6).

In claim 32, the terminal phosphate-labeled nucleoside polyphosphate has 4 or more phosphates in the polyphosphate chain, and the labeled polyphosphate is detected directly (i.e., without a phosphatase treatment). Different terminal-labeled nucleoside polyphosphates are used to identify additional sequences. (See page 6, lines 4-26). Again, the detection is effected without separation by charge of the labeled polyphosphate from the other reaction components (Page 13, lines 25-33).

Grounds of Rejection to be Reviewed on Appeal

1. Whether claims 1-56 are properly rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement.
2. Whether claims 1-7, 9, 11-18, 20-23, 27-38, 40, 42-45, 47, 49-50, 55 and 56 are properly rejected under 35 U.S.C. §102 (b) as being anticipated by Williams et al. (WO/2001/094609).
3. Whether claims 8 and 39 are properly rejected under 35 U.S.C. §103(a) as being unpatentable over Williams et al. in view of Wittwer et al (USPN

6,174,670).

4. Whether claims 10 and 41 are properly rejected under 35 U.S.C. §103(a) as being unpatentable over Williams et al. in view of Keller et al (USPN 5,656,462).
5. Whether claims 19 and 46 are properly rejected under 35 U.S.C. §103(a) as being unpatentable over Williams et al. in view of Lichenwalter et al (USPN 5,683,875).
6. Whether claims 23-25 are properly rejected under 35 U.S.C. §103(a) as being unpatentable over Williams et al. in view of Hattori et al (USPN 5,821,095).
7. Whether claims 25 and 26 are properly rejected under 35 U.S.C. §103(a) as being unpatentable over Williams et al. in view of Bronstein et al (USPN 5,112,960).

Argument

1. **Claims 1-56 are not properly rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement.**

The Examiner regards the claims as containing new matter due to the recitation in claim 1 of "without first separating by charge of said detectable species from the reaction mixture", and the recitation in claim 32 of "without first separating by charge of said labeled polyphosphate from the reaction mixture". The Examiner states that the specification does not appear to recite the limitations anywhere. Appellants respectfully disagree.

In response, Appellants submit that a skilled person provided with the specification would clearly understand that in the claimed methods, the labeled polyphosphate or detectable species does not need to be separated by charge prior to

detection. For example, the specification at page 4, lines 6-9, states, "The labeled polyphosphate then reacts with phosphatase or a phosphate or polyphosphate transferring enzyme to produce free label with a signal readily distinguishable from the phosphate bound dye". Appellants submit that the phrase "readily distinguishable" clearly indicates that no separation is required. Later in the same paragraph (page 4, lines 12-14), the specification states that, "After sufficient time is allowed for the polymerization reaction, which may range from milliseconds to several minutes, and detecting the presence or absence of signal, solid support may be separated from solution…". Again, Appellants submit that the description clearly indicates that no separation is required here prior to signal detection. Further, in describing the terminal-phosphate-labeled nucleotide, the label is clearly defined on page 12, lines 1-6, which again indicates that separation is not required of the current claimed methods.

Similarly, the specification provides support for the amendment to claim 32 as well. For example, the specification states that "in embodiments including terminal-phosphate-labeled nucleotides having four or more phosphates in the polyphosphate chain, it is within the contemplation of the present invention that the labeled polyphosphate by-product of phosphoryl transfer may be detected without the use of phosphatase treatment. ……Upon incorporation of the nucleoside monophosphate, the label polyphosphate by-product may be detected due to its enhanced fluorescence." (page 13, lines 25-33). Appellants submit that the specification taking as a whole, clearly describes sequencing methods which do not require a separation step, prior to the detecting step, of either the detectable species or the labeled polyphosphate from the reaction mixture.

Appellants submit that although the specification does not have the negative

limitation spelled out literally, it clearly suggests that no separation step is required. Taken as a whole, a skilled person would readily recognize that no matter is added by the amendments to claim 1 or 32. The new matter rejection of the claims can not be sustained and should be withdrawn.

2. Claims 1-7, 9, 11-18, 20-23, 27-38, 40, 42-45, 47, 49-50, 55 and 56 are not properly rejected under 35 U.S.C. §102 (b) as being anticipated by Williams et al. (WO/2001/094609).

As the Examiner points out, Williams et al. discloses a method for solid phase sequencing of a nucleic acid, which method includes the use of a labeled NP having a detectable moiety, wherein the detectable moiety is released as a charged detectable moiety when the NP is incorporated into the primer. After the application of an energy field, the charged detectable moiety is detected thereby the target nucleic acid sequenced (see e.g. page 4, lines 20-29). Williams et al. teaches that the NP is a nucleoside triphosphate, and the detectable moiety is a gamma-phosphate with a fluorophore attached (see e.g. page 4, lines 14-15). Williams et al. teaches that a phosphatase can be used to enhance "the charge-switch magnitude by dephosphorylating the PPi-F" (see e.g. page 25, lines 12-13).

The sequencing method of instant claim 1 includes the use of a terminal-phosphate-labeled nucleoside polyphosphate that is substantially non-reactive to phosphatase. The labeled polyphosphate produced from the polymerase reaction is treated with a phosphatase to generate a detectable species. This detectable species is subsequently detected without first separation by charge of the detectable species.

Appellants respectfully submit that while Williams et al. suggests the use of a phosphatase to enhance the charge-switch magnitude of the labeled polyphosphate,

the property/detectability of the label in Williams et al. remains the same before and after phosphatase treatment. The only change phosphatase treatment brings to the label in Williams et al. is a change in charge. In comparison, in claim 1 of the instant application, a detectable species is generated after phosphatase treatment. Appellants assert that this is not taught by Williams et al.

Appellants submit that Williams et al. requires a separation by charge of the dye-labeled pyrophosphate product of the polymerase reaction (or the phosphatase treated product, with enhanced charge-switch magnitude) from the dye-labeled nucleotides, prior to detection of the dye (see e.g. Figure 7). This is critical for Williams et al., as the detectable property of the dye (whether as part of the dye-labeled pyrophosphate or the phosphatase treated product) is the same as prior to the polymerase reaction. Without physical separation, it is impossible to distinguish the reaction product from the dye-labeled nucleotide. In claim 1 of the current invention, however, the detectable species generated by phosphatase treatment is readily distinguishable from untreated labeled polyphosphate, or labeled nucleotide, a separation of the dye product (detectable species) from the rest of the reaction components is not necessary. In fact, the labeled nucleotide in the current application is inert to phosphatase treatment, and therefore, phosphatase treatment can be carried out simultaneously with the polymerase reaction (see e.g. page 3, lines 17-31; claim 13).

Appellants submit that there is no teaching or suggestion in Williams et al. for a sequencing method represented by claim 1 of the instant application.

The sequencing method of instant claim 32 differs from that of claim 1 in that the terminal-phosphate-labeled nucleotide polyphosphate used here includes 4 or more phosphates. Thus the labeled polyphosphate produced from the polymerase

reaction is detected without the need of a phosphatase treatment. This is a simpler, but similar process compared to that of claim 1. The arguments above against Williams et al. apply to this claim as well.

Briefly, the property/detectability of the label in Williams et al. remains the same before and after the polymerase reaction. The only change the polymerase reaction brings to the label in Williams et al. is a change in net charge. In comparison, in claim 32 of the instant application, the labeled polyphosphate produced from the polymerase reaction is readily detectable. Appellants assert that this is not taught by Williams et al.

Appellants submit that Williams et al. require a separation by charge of the dye-labeled pyrophosphate product of the polymerase reaction from the dye-labeled nucleotides, prior to detection (see e.g. Figure 7). This is critical for Williams et al., as the detectable property of the dye is the same as prior to the polymerase reaction. Without physical separation, it is impossible to distinguish the reaction product from the dye-labeled nucleotide. In claim 32 of the current invention, however, the labeled polyphosphate is readily distinguishable from the labeled nucleotide, a separation of the labeled polyphosphate from the rest of the reaction components is not necessary.

Appellants submit that there is no teaching or suggestion in Williams et al. for a sequencing method represented by claim 32 of the instant application.

In the Advisory Action, the Examiner states that Williams et al. does present a method that does not recite a step of separation based on charge on page 24, lines 15-26. The Examiner further states that Williams et al. also teach separation of the cleaved phosphate dye by diffusion, on page 20, lines 20-25. Appellants submit that the Examiner mischaracterizes the reference.

Appellants submit that the Examiner apparently reads something into the

Williams et al. reference. It is submitted that nothing in the section of Williams et al. on page 24, lines 15-26 suggests the method could be performed without a step of separation by charge. Appellants submit that the cited method clearly uses "a charge-switch NTP", having a fluorophore attached to the gama-phosphate. This implies that the method includes a step of separation by charge. This is because the fluorophore label, whether part of the nucleotide or released from the nucleotide, has the same fluorescent property therefore can not be differentiated without being physically separation. Appellants submit that unlike the Examiner's assertion, the teachings of Williams et al. on page 24 do imply separation by charge, although it was not explicitly stated. Williams et al. clearly states that "a charge-switch NTP" was used, and therefore a separation step based on charge is part of the method.

With regard to the section on page 20, lines 20-25 of Williams et al., Appellants first submit that the Examiner apparently meant to refer to the section on page 21, lines 20-25, where the term "diffuse" was mentioned. This section, however, clearly states that the PPi-Dye, after diffusion into the medium, is directed by a transverse electric field toward the negative electrode. The Ppi-Dye is detected in the transverse channel (Figure 7). Again, a separation step based on charge is part of the method.

In summary, Appellants submit that the rejection of claims 1 and 32 over Williams et al. should be withdrawn. Therefore the 35 U.S.C. §102 (b) rejection of claims 1-7, 9, 11-18, 20-23, 27-38, 40, 42-45, 47, 49-50, 55 and 56 should also be withdrawn.

3. Claims 8 and 39 are not properly rejected under 35 U.S.C. §103(a) as being unpatentable over Williams et al. in view of Wittwer et al. (US 6,174,670).

The Examiner has rejected claims 8 and 39 under 35 U.S.C. §103(a), as being unpatentable over Williams et al. in view of Wittwer et al. Appellants do not agree. As stated above, Williams et al. does not disclose or even suggest the independent claims 1 or 32, upon which claims 8 and 39 depend, respectively. In view of this, Appellants submit that the §103(a) rejections of claims 8 and 39 over Williams et al. in view of Wittwer et al. should be withdrawn.

4. Claims 10 and 41 are not properly rejected under 35 U.S.C. §103(a) as being unpatentable over Williams et al. in view of Keller et al. (US 5,656,462).

The Examiner has rejected claims 10 and 41 under 35 U.S.C. §103(a), as being unpatentable over Williams et al. in view of Keller et al. Appellants do not agree. As stated above, Williams et al. does not disclose or even suggest the independent claims 1 or 32, upon which claims 10 and 41 depend, respectively. In view of this, Appellants submit that the §103(a) rejections of claims 10 and 41 over Williams et al. in view of Keller et al. should be withdrawn.

5. Claims 19 and 46 are not properly rejected under 35 U.S.C. §103(a) as being unpatentable over Williams et al. in view of Lichenwalter et al. (US 5,683,875).

The Examiner has rejected claims 19 and 46 under 35 U.S.C. §103(a), as being unpatentable over Williams et al. in view of Lichtenwalter et al. Appellants do not agree. As stated above, Williams et al. does not disclose or even suggest the independent claims 1 or 32, upon which claims 19 and 46 depend, respectively. In view of this, Appellants submit that the §103(a) rejection of claims 19 and 46 over Williams et al. in view of Lichtenwalter et al. should be withdrawn.

6. Claims 23-25 are not properly rejected under 35 U.S.C. §103(a) as being unpatentable over Williams et al. in view of Hattori et al. (US 5,821,095).

Claims 23-25 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Williams in view of Hattori et al. Appellants do not agree. As stated above, Williams et al. does not disclose or even suggest independent claim 1, upon which these claims depend. In view of this, Appellants submit that the 35 U.S.C. §103(a) rejections of claims 23-25 should be withdrawn.

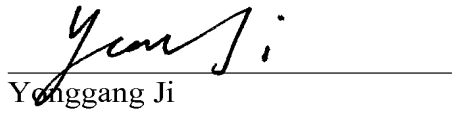
7. Claims 25 and 26 are not properly rejected under 35 U.S.C. §103(a) as being unpatentable over Williams et al. in view of Bronstein et al. (US 5,112,960).

Claims 25 and 26 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Williams et al. in view of Bronstein et al. Appellants do not agree. As stated above, Williams et al. does not disclose or even suggest independent claim 1, upon which these claims depend. In view of this, Appellants submit that the 35 U.S.C. §103(a) rejections of claims 25 and 26 should be withdrawn.

Conclusion

In view of the foregoing arguments, Appellants respectfully assert that the Examiner's rejections cannot be sustained and should be reversed.

Respectfully submitted,



Yonggang Ji
Registration No.: 53,073
Agent for Appellants

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue
P. O. Box 1327
Piscataway, New Jersey 08855-1327

Tel: (732) 980-2875
Fax: (732) 457-8463

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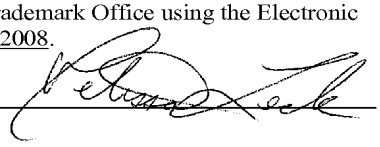
Signature: 

Name: Melissa Leck

CLAIMS APPENDIX

The Rejected Claims

Claim 1 (previously presented): A method of sequencing a target region of a nucleic acid template, comprising:

a) conducting a nucleic acid polymerization reaction on a solid support, by forming a reaction mixture, said reaction mixture including a nucleic acid template, a primer, a nucleic acid polymerizing enzyme, and one terminal-phosphate-labeled nucleoside polyphosphate which is substantially non-reactive to phosphatase, said nucleoside polyphosphate is selected from a nucleoside with a natural base or a base analog

wherein a component of said reaction mixture or complex of two or more of said components, is immobilized on said solid support, and said component or components are selected from the group consisting of said nucleic acid template, said primer, and said nucleic acid polymerizing enzyme,

and

said reaction results in production of labeled polyphosphate if said terminal-phosphate-labeled nucleoside polyphosphate contains a base complementary to the template base at the site of polymerization;

b) subjecting said reaction mixture to a phosphatase treatment to produce a detectable species if said labeled polyphosphate is produced in step a);

c) detecting said detectable species without first separating by charge of said detectable species from the reaction mixture;

d) continuing said polymerization reaction by adding a different terminal-phosphate-labeled nucleoside polyphosphate selected from the remaining natural bases or base analogs to said reaction mixture and repeating steps b and c; and

e) identifying said target region sequence from the identity and order of addition of terminal-phosphate labeled nucleoside polyphosphates resulting in production of said detectable species.

Claim 2 (original): The method of claim 1, wherein said nucleic acid template is immobilized on said solid support in said conducting step.

Claim 3 (original): The method of claim 1, wherein said primer is immobilized on said solid support in said conducting step.

Claim 4 (original): The method of claim 1, wherein said nucleic acid template and said primer are first hybridized and then immobilized on said solid support in said conducting step.

Claim 5 (original): The method of claim 1, wherein said nucleic acid polymerization enzyme is immobilized on said solid support in said conducting step.

Claim 6 (original): The method of claim 1, wherein said steps are carried out in a sequential manner in a flow through or a stop-flow system.

Claim 7 (original): The method of claim 1, further comprising the step of quantifying said nucleic acid sequence.

Claim 8 (original): The method of claim 1, further comprising: quantifying said nucleic acid sequence by comparing spectra produced by said detectable species with a spectra produced from a known standard.

Claim 9 (original): The method of claim 1, wherein said nucleic acid polymerizing enzyme is a polymerase.

Claim 10 (original): The method of claim 1, wherein said nucleic acid template is an RNA template.

Claim 11 (original): The method of claim 1, wherein said nucleic acid template is a DNA template.

Claim 12 (original): The method of claim 1, wherein said nucleic acid template is a natural or synthetic oligonucleotide.

Claim 13 (original): The method of claim 1, wherein said conducting step and said subjecting step are carried out simultaneously.

Claim 14 (original): The method of claim 1, wherein said terminal phosphate-labeled nucleoside polyphosphate comprises four or more phosphate groups in the polyphosphate chain.

Claim 15 (original): The method of claim 1, wherein said detectable species is produced in amounts substantially proportional to the amount of nucleic acid sequence.

Claim 16 (original): The method of claim 1, wherein said phosphatase is an acid phosphatase, an alkaline phosphatase or another phosphate transferring enzyme.

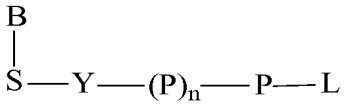
Claim 17 (original): The method of claim 1, further comprising including one or more additional detection reagents in said polymerization reaction.

Claim 18 (original): The method of claim 17, wherein said one or more additional detection reagents are each independently, capable of a response that is detectably different from each other and from said detectable species.

Claim 19 (original): The method of claim 17, wherein one or more of said one or more additional detection reagents is an antibody.

Claim 20 (original): The method of claim 1, wherein said detectable species is detectable by a property selected from the group consisting of color, fluorescence emission, chemiluminescence, mass change, reduction/oxidation potential and combinations thereof.

Claim 21 (original): The method of claim 1, wherein said terminal-phosphate-labeled nucleoside polyphosphate is represented by the formula:



wherein

P is phosphate (PO_3) and derivatives thereof;

n is 2 or greater;

Y is an oxygen or sulfur atom;

B is a nitrogen-containing heterocyclic base;

S is an acyclic moiety, carbocyclic moiety or sugar moiety;

P-L is a phosphorylated label which becomes independently detectable when the phosphate is removed,

wherein L is an enzyme-activatable label containing a hydroxyl group, a sulfhydryl group or an amino group suitable for forming a phosphate ester, a thioester or a phosphoramidate linkage at the terminal phosphate of a natural or modified nucleotide.

Claim 22 (original): The method of claim 21, wherein said enzyme-activatable label is selected from the group consisting of chemiluminescent compounds, fluorogenic dyes, chromogenic dyes, mass tags, electrochemical tags and combinations thereof.

Claim 23 (original): The method of claim 22, wherein said fluorogenic dye is selected from the group consisting of 2-(5'-chloro-2'-phosphoryloxyphenyl)-6-chloro-4-(3H)-quinazolinone, fluorescein diphosphate, fluorescein 3'(6')-*O*-alkyl-6'(3')-phosphate, 9H-(1,3-dichloro-9,9-dimethylacridin-2-one-7-yl)phosphate, 4-methylumbelliferyl phosphate, resorufin phosphate, 4-trifluoromethylumbelliferyl phosphate, umbelliferyl

phosphate, 3-cyanoumbelliferyl phosphate, 9,9-dimethylacirdin-2-one-7-yl phosphate, 6,8-difluoro-4-methylumbelliferyl phosphate, and derivatives thereof.

Claim 24 (original): The method of claim 22, wherein said chromogenic dye is selected from the group consisting of 5-bromo-4-chloro-3-indolyl phosphate, 3-indoxyl phosphate, p-nitrophenyl phosphate and derivatives thereof.

Claim 25 (original): The method of claim 22, wherein said chemiluminescent compound is a phosphatase-activated 1, 2-dioxetane compound.

Claim 26 (original): The method of claim 25, wherein said 1,2-dioxetane compound is selected from the group consisting of 2-chloro-5-(4-methoxyspiro[1,2-dioxetane-3,2'-(5-chloro-)tricyclo[3,3,1-1^{3,7}]-decan]-1-yl)-1-phenyl phosphate, chloroadamant-2'-ylidenemethoxyphenoxy phosphorylated dioxetane, 3-(2'-spiroadamantane)-4-methoxy-4-(3''-phosphoryloxy)phenyl-1,2-dioxetane and derivatives thereof.

Claim 27 (original): The method of claim 21, wherein said sugar moiety is selected from the group consisting of ribosyl, 2'-deoxyribosyl, 3'-deoxyribosyl, 2', 3'-dideoxyribosyl, 2', 3'-didehydrodideoxyribosyl, 2'-alkoxyribosyl, 2'-azidoribosyl, 2'-aminoribosyl, 2'-fluororibosyl, 2'-mercaptoriboxyl, 2'-alkylthioribosyl, carbocyclic, acyclic and other modified sugars.

Claim 28 (original): The method of claim 21, wherein said sugar moiety is selected from ribosyl or 2'-deoxyribosyl sugar.

Claim 29 (original): The method of claim 21, wherein said nitrogen-containing heterocyclic base is selected from the group consisting of uracil, thymine, cytosine, 5-methylcytosine, guanine, 7-deazaguanine, hypoxanthine, 7-deazahypoxanthine, adenine, 7-deazaadenine, 2,6-diaminopurine and analogs thereof.

Claim 30 (original): The method of claim 1, wherein said target region of a nucleic acid template has a known sequence and wherein the order of addition of terminal-phosphate labeled nucleoside polyphosphates is based on the sequence of the target region.

Claim 31 (original): The method of claim 1, wherein said target region of a nucleic acid template has an unknown sequence and wherein the order of addition of terminal-phosphate labeled nucleoside polyphosphates occurs in a preset cycle, said preset cycle being repeated without regard to the identity of the terminal-phosphate labeled nucleoside polyphosphates incorporated in a given cycle.

Claim 32 (previously presented): A method of sequencing a target region of a nucleic acid template, comprising:

a) conducting a nucleic acid polymerization reaction on a solid support, by forming a reaction mixture, said reaction mixture including a nucleic acid template, a primer, a nucleic acid polymerizing enzyme, and one terminal-phosphate-labeled nucleoside polyphosphate with 4 or more phosphates which nucleoside polyphosphate is substantially non-reactive to phosphatase, and said nucleoside polyphosphate is selected from a nucleoside with a natural base or a base analog and

wherein a component of said reaction mixture or a complex of two or more of said components, is immobilized on said solid support, and said component or components are selected from the group consisting of said nucleic acid template, said primer, and said nucleic acid polymerizing enzyme,

and

said reaction results in production of labeled polyphosphate if said terminal-phosphate-labeled nucleoside polyphosphate contains a base complementary to the template base at the site of polymerization;

b) detecting said labeled polyphosphate without first separating by charge of said labeled polyphosphate from the reaction mixture;

c) continuing said polymerization reaction by adding a different terminal-phosphate-labeled nucleoside polyphosphate selected from the remaining natural bases or base analogs to said reaction mixture and repeating step b; and

d) identifying said target region sequence from the identity and order of addition of terminal-phosphate labeled nucleoside polyphosphates resulting in production of said labeled polyphosphates.

Claim 33 (original): The method of claim 32, wherein said nucleic acid template is immobilized on said solid support in said conducting step.

Claim 34 (original): The method of claim 32, wherein said primer is immobilized on said solid support in said conducting step.

Claim 35 (original): The method of claim 32, wherein said nucleic acid template and said primer are first hybridized and then immobilized on said solid support in said conducting step.

Claim 36 (original): The method of claim 32, wherein said nucleic acid polymerization enzyme is immobilized on said solid support in said conducting step.

Claim 37 (original): The method of claim 32, wherein said steps are carried out in a sequential manner in a flow through or a stop-flow system.

Claim 38 (original): The method of claim 32, further comprising the step of quantifying said nucleic acid sequence.

Claim 39 (original): The method of claim 32, further comprising: quantifying said nucleic acid sequence by comparing spectra produced by said detectable species with a spectra produced from a known standard.

Claim 40 (original): The method of claim 32, wherein said nucleic acid polymerizing enzyme is a polymerase.

Claim 41 (original): The method of claim 32, wherein said nucleic acid template is an RNA template.

Claim 42 (original): The method of claim 32, wherein said nucleic acid template is a DNA template.

Claim 43 (original): The method of claim 32, wherein said nucleic acid template is a natural or synthetic oligonucleotide.

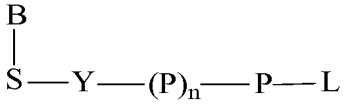
Claim 44 (original): The method of claim 32, further comprising including one or more additional detection reagents in said polymerization reaction.

Claim 45 (original): The method of claim 44, wherein said one or more additional detection reagents are each independently, capable of a response that is detectably different from each other and from the said labeled polyphosphate.

Claim 46 (original): The method of claim 44, wherein one or more of said one or more additional detection reagents is an antibody.

Claim 47 (original): The method of claim 32, wherein said labeled polyphosphate is detectable by a property selected from the group consisting of color, fluorescence emission, mass change, reduction/oxidation potential and combinations thereof.

Claim 48 (original): The method of claim 32, wherein said terminal-phosphate-labeled nucleoside polyphosphate is represented by the formula:



wherein

P is phosphate (PO_3) and derivatives thereof;

n is 3 or greater;

Y is an oxygen or sulfur atom;

B is a nitrogen-containing heterocyclic base;

S is an acyclic moiety, carbocyclic moiety or sugar moiety; and

P-L is a phosphorylated label,

wherein L is a label containing a hydroxyl group, a haloalkyl group, a sulfhydryl group or an amino group suitable for forming a phosphate ester, a phosphonate, a thioesteror a phosphoramidate linkage at the terminal phosphate of a natural or modified nucleotide.

Claim 49 (original): The method of claim 48, wherein said label is selected from the group consisting of fluorescent dyes, colored dyes, mass tags, electrochemical tags and combinations thereof.

Claim 50 (previously presented): The method of claim 49, wherein said fluoroscent dye is selected from the group consisting of a xanthene dye, a cyanine dye, a merrocyanine dye, an azo dye, a porphyrin dye, a coumarin dye, a bodipy dye and derivatives thereof.

Claim 51 (original): The method of claim 49, wherein said colored dye is selected from the group consisting of an azo dye, a merrocyanine, a cyanine dye, a xanthene dye, a porphyrin dye, a coumarin dye, a bodipy dye and derivatives thereof.

Claim 52 (original): The method of claim 48, wherein said sugar moiety is selected from the group consisting of ribosyl, 2'-deoxyribosyl, 3'-deoxyribosyl, 2', 3'-dideoxyribosyl, 2', 3'-didehydrodideoxyribosyl, 2'-alkoxyribosyl, 2'-azidoribosyl, 2'-

aminoribosyl, 2'-fluororibosyl, 2'-mercaptoriboxyl, 2'-alkylthioribosyl, carbocyclic, acyclic and other modified sugars.

Claim 53 (original): The method of claim 48, wherein said sugar moiety is selected from ribosyl or 2'-deoxyribosyl sugar.

Claim 54 (original): The method of claim 48, wherein said nitrogen-containing heterocyclic base is selected from the group consisting of uracil, thymine, cytosine, 5-methylcytosine, guanine, 7-deazaguanine, hypoxanthine, 7-deazahypoxanthine, adenine, 7-deazaadenine, 2,6-diaminopurine and analogs thereof.

Claim 55 (original): The method of claim 32, wherein said target region of a nucleic acid template has a known sequence and wherein the order of addition of terminal-phosphate labeled nucleoside polyphosphates is based on the sequence of the target region.

Claim 56 (original): The method of claim 32, wherein said target region of a nucleic acid template has an unknown sequence and wherein the order of addition of terminal-phosphate labeled nucleoside polyphosphates occurs in a preset cycle, said preset cycle being repeated without regard to the identity of the terminal-phosphate labeled nucleoside polyphosphates incorporated in a given cycle.

Claims 57-62 (cancelled)

EVIDENCE APPENDIX

Appellants hereby append:

1). Williams et al. WO/2001/094609.

2). Wittwer et al. USPN 6,174,670.

3). Keller et al. USPN 5,656,462.

4). Lichenwalter et al. USPN 5,683,875.

5). Hattori et al. USPN 5,821,095.

6). Bronstein et al. USPN 5,112,960.

These are the evidence relied upon by the Examiner for rejection of appealed claims.

RELATED PROCEEDINGS APPENDIX

There are no other appeals or interferences related to the instant appeal.